Biomolecular NMR Spectroscopy

904-Pos
Characterization and optimization of Nonuniform Sampling for Multidimensional NMR Experiments
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Nuclear magnetic resonance (NMR) is a powerful technique for revealing biomolecular structure and dynamics. Technical advances over the past decade, including cryogenic probes, higher magnetic fields, and novel pulse sequences, allow for the analysis of increasingly larger and more complex systems. Multidimensional, multinuclear experiments are invariably required to resolve individual resonances. However, the increased sampling rate imposed by the Nyquist theorem at higher magnetic fields (due to greater spectral dispersion) means that experiment times become prohibitively long when conventional uniform sampling is employed. Non-Fourier methods of spectrum analysis open the possibility of nonuniform sampling, permitting data to be collected at short evolution times to ensure sensitivity and at long evolution times to allow for high resolution. The combination of nonuniform sampling methods with non-Fourier methods of spectrum analysis enable the computation of high resolution multidimensional spectra from much shorter data records than can be employed using conventional Fourier and uniform sampling methods. The design of optimal sampling strategies, for minimizing sampling time while maintaining resolution and sensitivity, or optimizing sensitivity and/or resolution for a given total experiment time remains an open challenge.
In the present work the maximum entropy (MaxEnt) method of spectrum reconstruction is used to characterize the performance of nonuniform sampling strategies with respect to sensitivity and resolution. A peak identification algorithm is developed along with a metric for spectra comparison. These tools are used to investigate whether prior knowledge of peak frequencies can aid the design of optimal sampling strategies. Such strategies could be useful in a number of contexts in biomolecular NMR, including relaxation studies and multidimensional experiments conducted subsequent to ''scout'' experiments, such as two-dimensional HSQC.

905-Pos
Enabling Site-Resolved Measurement of Hydration Water-Protein Interactions by Solution NMR
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The interaction between a protein and solvating water is fundamental to its stability, dynamics, and function. In principle, solution NMR methods can be employed to identify and characterize the location and dynamics of specific solvent-protein interactions. Unfortunately, the residence times of water on the surface of a protein in bulk solution are such that the elegant experiments of Wà1/4thrich and coworkers are defeated. Here, we take advantage of the slowed dynamics of water under nanoconfinement and tap the full potential of these experiments to obtain the first site-resolved, solution NMR measurements of hydration water-protein interactions. Reverse micellar encapsulation slows water dynamics enough to allow detectable build-up of direct protein-water dipolar magnetization exchange while at the same time slowing hydrogen exchange kinetics by up to two orders of magnitude, thereby reducing indirect contributions to the spectra. Using amide hydrogens as a probe, we are able to detect literally dozens of hydration waters on the surface of encapsulated ubiquitin. Three-dimensional ^{15}N-NOESY and ROESY spectra of ubiquitin in reverse micelles have been obtained. The relative intensities of NOE/ROE cross peaks at the water resonance confirms that a minority of the detected hydration waters have long residence times ($\omega\tau > 1$), while the majority of detected solvation waters have a range of shorter residence times. This approach appears to open the door to global site-resolved descriptions of protein hydration dynamics and therefore represent a major step forward in the quest to understand the motional links between proteins and solvent. Supported by NSF-0842814 and NIH F32GM074376 (M.P.) and F32GM08709 (N.N.).

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Dynamics of Retinal Studied by ^{2}H NMR Relaxation Underlie Multiscale Conformational Changes in Rhodopsin Activation
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The activation mechanism of the G protein-coupled receptor rhodopsin has been investigated through solid-state ^{2}H NMR relaxation studies. Rhodopsin was regenerated with retinal ^{2}H-labeled at the C5-, C9-, and C13-methyl groups and was recombined into POPC (dark state and Meta I) or POPC:DOPE (Meta II) [1-4]. The ^{2}H NMR relaxation rates of Zeeman order (R_{1Z}) or quadrupolar order (R_{1Q}) were measured from -30 to -150°C in the dark, Meta I, and Meta II states. Relaxation data were interpreted in terms of axial rotation and off-axial motion of the methyl groups and revealed interactions between the retinal cofactor and the rhodopsin binding pocket. Relaxation rates show considerable mobility of the retinal ligand in the dark state despite the complete absence of basal activity. ^{2}H NMR data indicate that the retinal C9-methyl group does not change its orientation upon photoisomerization. Rather it acts as a hinge for light-induced rotation of the C13-methyl toward the second extracellular loop E2. The C13-methyl rotation leads to a displacement of the E2 loop that is observed already in the Meta I state. Short T_{1Z} relaxation times for the C5-methyl indicate that the β-ionone ring adopts a twisted 6-*s*-*cis* conformation, and remains in its hydrophobic binding pocket up to the Meta II state. An activation mechanism is proposed whereby the photonic energy is channeled by 11-*cis* to *trans* isomerization of retinal against the E2 loop by the C9- and C13-methyl groups, and toward helices H5 and H3 by the β-ionone ring and C5-methyl group.[1] G.F.J. Salgado *et al.* (2004) *Biochemistry* **43**, 12819. [2] G.F.J. Salgado *et al.* (2006) *JACS***128**, 11067. [3] A.V. Struts *et al.* (2007) *JMB***372**, 50. [4] M.F. Brown *et al.* (2009) *Photochem. Photobiol.* **85**, 442.

907-Pos
The Influence of Zn2+ on the Global Structure of the Prion Protein
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The prion protein (PrP) is implicated in the fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). These illnesses are unique because a cellular form of PrP misfolds to create an infectious agent, PrP^{Sc}. All birds and mammals produce PrP, however, very few naturally develop TSEs. Despite the fact that PrP is conserved among these species, little is known about its normal physiological function. Growing evidence suggests that PrP binds both copper and zinc *in vivo*. These metals induce PrP endocytosis, inhibit *in vitro* fibril formation, and promote intermolecular interactions. Metal binding occurs in the flexible and unstructured N-terminal half of PrP. Zinc binding is restricted to the repeat region known as the octarepeats (PHGGGWGQ), in which the four histidines from the octarepeat domain coordinate a single Zn^{2+} atom. The structured C-terminal domain is the proposed initiation site of the conversion to PrP^{Sc}. The global structure of PrP bound to Zn^{2+} is unknown, and in particular, whether metal ion coordination influences the structured C-terminal domain. To study the potential interaction of the N- and C- termini upon Zn^{2+} binding, we created ^{15}N labeled mouse PrP for NMR studies. We titrated Zn^{2+} into the protein and examined the changes in the HSQC spectra. The results from this study will help to determine whether Zn^{2+} plays a protective or harmful role in the progression to prion diseases. Furthermore, it might lend insight into the physiological function of PrP.

908-Pos
Mechanisms of Molecular Recognition by the Transcription Factor LMO7
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Recent results with the characterization of the structural details and the interactions of the transcription factor LIM domain only-7 (LMO7) protein will be presented. LMO7 has a role in stabilizing the communication between the cadherin and nectin associated complexes in the cell-to-cell adherens junctions through its association with α-actinin and afadin. In the nucleus, LMO7 interacts at the nuclear envelope with the protein emerin, and also regulates the transcription of genes important for heart, muscle and retina formation. The structure-function relationships associated with the molecular recognition processes of two domains within LMO7 are being studied using Nuclear Magnetic Resonance (NMR) spectroscopy, molecular biological, biochemical and biophysical methods. Three dimensional structures of these domains and characterization of their mechanism of molecular recognition will be discussed.